CYTOLOGY OF GEOTRICHUM VERSIFORME MOORE[1]

MORRIS MOORE

Mycologist to The Barnard Free Skin and Cancer Hospital, St. Louis

Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of Washington University

INTRODUCTION

The purpose of this paper is to report a study of the cytology and nuclear phenomena in the development of *Geotrichum versiforme,* a fungus which was described morphologically and culturally in a previous report by the author (Moore, '34).

As far as the writer is aware, there has been no cytological study of the genus *Geotrichum,* except for the suggestion that the arthrospores were possibly uninucleate cells. Guilliermond ('00), in describing the structure of *Oidium lactis,* gave indications of the mycelial structure of that organism, while Jannin ('13) referred to the cytology in Guilliermond's paper as representing the type present in Mycodermata. Inasmuch as *Geotrichum* has a structure and development often confused with and similar to *Mycoderma, Oidium,* and perhaps *Oospora* and *Monilia,* the work of the above two writers may be taken to represent the previous work on the subject. This may further be emphasized by the fact that certain Oidia, Mycodermata, and others have been shown to be synonymous. However, the life cycle of *Geotrichum versiforme* seemed, on superficial examination, to differ in several factors from that of the other genera. In addition, a newer technique for work of this sort led the author to the study here described.

MATERIALS AND TECHNIQUE

It has been shown that the fungus has different aspects on various media, and that maltose increased the growth of the

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organism. Consequently, Sabouraud's glucose and maltose agar were used as substrates. The former develops all the features found in the life cycle, and the latter, because of the maltose, produced many of the irregularities in morphology, such as large sclerotic cells. Cultures on agar slants of these media were fixed with Hermann's fluid, embedded in celloidin, sectioned, and stained with iron-alum haematoxylin as described by the author (Moore, '33) for *Endomyces*.

For the observation of the cellular constituents, as fat, glycogen, volutin, and the like, several chemicals were used which will be described under their respective headings.

CYTOLOGY

Since there has been no definite relationship established with the Ascomycetes, by means of ascospores, the arthrospore must be considered as the germ of the colony. It would thus seem advisable to trace the development of a mycelium with that cell as the initial stage. The single arthrospore (pl. 7, fig. 1) is uninucleate, with a fairly thick wall or membrane. Germination proceeds, usually laterally, by sending out a thin tube, the germ-tube, which at first has the appearance of a small bud but later becomes a thin-walled hypha. This contains many granules near its apex which are probably volutin. The nucleus is seen as a large granular structure with a heavily staining nucleolus. With the germination of the arthrospore, nuclear division takes place, by simple fission as far as could be determined (pl. 7, fig. 3). Although many nuclear divisions were examined, it cannot be stated definitely that mitosis was present. The process continues until there may be as many as eight nuclei in the germinating cell, but usually there are only two or three (pl. 7, fig. 2).

The germ cell itself contains a granular and reticulated protoplasm of metachromatic material. In many of the germ-tubes, there seems to be a vacuolated area near the apex (pl. 7, fig. 3). After a number of nuclei have apparently entered into the germ-tube, the hypha is ready to form cross-walls. In the young filaments this is accomplished by a pinching in or a transverse abscission of the walls of the hypha. This may take

place between a dividing nucleus as in pl. 7, fig. 4, or between separated nuclei. The cell elongates and nuclear division continues, chiefly at the terminal portion. The nuclei may divide laterally (pl. 7, figs. 6, 8), giving the appearance in a later stage of alternate nuclei (pl. 7, figs. 8, 11, 12), or longitudinally (pl. 7, figs. 9, 12). The young filaments elongate and may be simple or branched (pl. 7, figs. 8–12; pl. 8, fig. 22) as explained in the previous paper (Moore, '34). When these become mature, cross-walls are again laid down, but at this time they are formed by a thickening of the region where the partition is to develop (pl. 7, figs. 9–10). These may be simple or collar-like (pl. 8, figs. 22, 24). With their formation, the resulting cells become uninucleate (pl. 7, figs. 13, 16) and are now the arthrospores. In addition to the reticulated network in the cell noted previously, there is a heavy granulation on the inner surface of the wall (pl. 7, figs. 16, 18; pl. 8, fig. 24) which seems to be noticed more often in the arthrospores.

An important feature in the filaments is the presence of clear, non-granulated, thin-walled cells, apparently devoid of cytoplasm (pl. 7, fig. 19; pl. 8, figs. 20–22, 24, 26–27, 32, 48, 53). What this lack of protoplasm indicates cannot be explained unless it is that the contents were used up in the nourishment of the adjoining cells.

On maltose agar the cells are much enlarged, especially those submerged. Here the peculiar condition arises of many long hyphae devoid of protoplasm with clusters of short branches of arthrospores as in pl. 8, fig. 22. Also, there are series of empty cells and arthrospores (pl. 8, fig. 24). These intercalary arthrospores give rise to chains of small arthrospores while still attached to the filament.

In addition to the regular development of the fungus, there are several structures which must be given consideration. The first of these is the chlamydospore. This particular organ which can generally be distinguished by its apparent large size and thick wall is found here (pl. 7, figs. 7, 17, 19; pl. 8, figs. 20–21, 23) in much the same condition as the arthrospores. It is a coenocytic structure which has a heavily granulated, reticular network and may occur terminally as a spherical body.

They may be found as spherical, cylindrical, intercalary structures (pl. 8, figs. 27, 48, 53) or in chains as sclerotic, thick-walled cells (pl. 7, fig. 19; pl. 8, figs. 20–21). Chlamydospores germinate (pl. 7, figs. 7, 17) and give rise to mycelium.

The so-called blastospores are seen as granulated, nucleated cells, the nuclei varying in number from one to three, frequently one. The conidia, considered by many to be possibly blastospores, have been found to have a heavily granulated protoplasm with no indication of a nucleus. A nucleus if present would be masked probably by the accumulated, heavily stained material within the cell. All indications, however, point to the absence of a nucleus, which would therefore justify the retention of the term conidium for that cell, as has been pointed out by the author.

Cellular contents.—Not all the constituents of the cell have been identified, but those that have will be considered here. It has been shown by many authors that fungi tend to store food in the cell, particularly in the older mycelium. These reserve products are usually in the form of glycogen, lipoids, oil globules, metachromatic granules of Guilliermond or nuclear decomposition products, as volutin, nucleic acid substances, and probably other protein derivatives and carbohydrates. Since most of these substances have been demonstrated and discussed by the author for *Endomyces*, it will suffice to mention here merely their presence and quantity and the technique employed.

Volutin.—Volutin or metachromatic material is very easily demonstrated with methylene blue or even iron-alum haematoxylin, as substances within the cell, usually along the inner surface of the cell wall (pl. 7, figs. 16, 18) or along the reticulated network. A pinch of benzidine sulphate added to a water mount of living material reveals a number of granules (pl. 8, figs. 33–41), particularly the so-called "dancing bodies" which are precipitated volutin in a state of Brownian movement. These take a blue coloration. According to the work of Bertrand (cited in Guilliermond, Mangenot and Plantefol, '33), benzidine produces with peroxidases a blue coloration.

Glycogen.—With neutral red and iodine potassium iodide, glycogen may be easily demonstrated. Neutral red shows this material as large, pink to red drops (pl. 8, figs. 25–27). The older cells have larger and more deeply colored drops than do the younger cells. With saturated iodine potassium iodide (pl. 8, figs. 28–32) glycogen is seen as orange-brown, irregular bodies spread throughout the cell. As in the case of neutral red, there is a greater amount in the older than in the younger cells.

Vacuoles.—The presence of vacuoles is easily demonstrated with methylene blue or haematoxylin, as surrounded by the reticulum (pl. 7, fig. 19). Iodine potassium iodide (pl. 8, figs. 28, 31) shows the vacuoles very clearly, while benzidine sulphate (pl. 8, figs. 36–41), as used previously, shows them to best advantage, most of them containing the "dancing bodies." Neutral red also brings out the vacuoles.

Chondriosomes.—Chondriosomes or mitochondria may be demonstrated with iodine potassium iodide. They are seen as light yellow, refractile droplets of various sizes. With benzidine sulphate they are supposed to take a light blue color and are distributed as droplets throughout the cell (pl. 8, figs. 33–35), and where vacuoles are present surround the vacuolar membrane (pl. 8, figs. 36, 40). Guilliermond has demonstrated them in *Oidium lactis* with benzidine. These bodies are few in young cells and abundant in older cells.

Fat, lipoidal substances.—In addition to the substances listed above, lipoidal substances, fats, and various other reserve materials, as well as secretion and excretion materials, can be demonstrated easily. They are found in varying amounts in the cells. These have also been discussed previously by the author.

Several agents can be used very favorably to study these materials, each showing some degree of difference. With 2 per cent osmic acid (pl. 8, figs. 42–48) these substances are reduced and take a black coloration. They are found as small globules or droplets in the young mycelium (pl. 8, fig. 47) and as larger, heavier masses usually in the center of the older cells. Much

the same picture is presented with 5 per cent platinic chloride solution (pl. 8, figs. 49–53). With iodine potassium iodide as applied for glycogen and chondriosomes, fats and lipoidal substances are seen, with careful focusing, as very small, highly refractile and hyaline bodies.

DISCUSSION AND CONCLUSIONS

It is not intended in this paper to formulate any new theories as to cytological pictures, but merely to interpret the phenomena involved in the development of *Geotrichum versiforme*. To summarize briefly, the uninucleate arthrospore serves as the spore for new mycelia. It germinates, forming a thin-walled tube which becomes multinucleate and when well elongated develops cross-walls to form coenocytic cells. These cells mature and secondary partitions are formed by a thickening and subsequent gelatinization of a particular portion of the hypha. This process therefore gives rise to cells which are the uninucleate arthrospores.

The division of the nucleus, as has been pointed out previously, is apparently direct or amitotic and may take place longitudinally or transversely in the cell. In the former case, cross-walls can be seen forming at the point of division, while in the latter the nuclei appear alternately on the sides of the hypha, due to the growth of the filament. Nuclear phenomena in *Oidium lactis* as described by Guilliermond ('00) are revealed by simple division into two masses within an areola. Dangeard, Janssens and Leblanc, and a host of others cited by the present author considered this division as mitotic, with the phenomena masked. In *Geotrichum*, as observed here, the light region or areola surrounding the nucleus is lacking. Darkly staining central portions may be seen which represent the nucleoli, but definite karyokinesis with chromosomal formation cannot be distinguished.

Chlamydospores are found as coenocytic, enlarged, sclerotic, thick-walled cells. So-called blastospores are present as nucleated structures, while granular, apparently non-nucleated conidia can be distinguished.

Volutin, glycogen vacuoles, chondriosomes, and additional storage or reserve materials, as well as fat and lipoidal substances, can be demonstrated in varying amounts by means of several agents.

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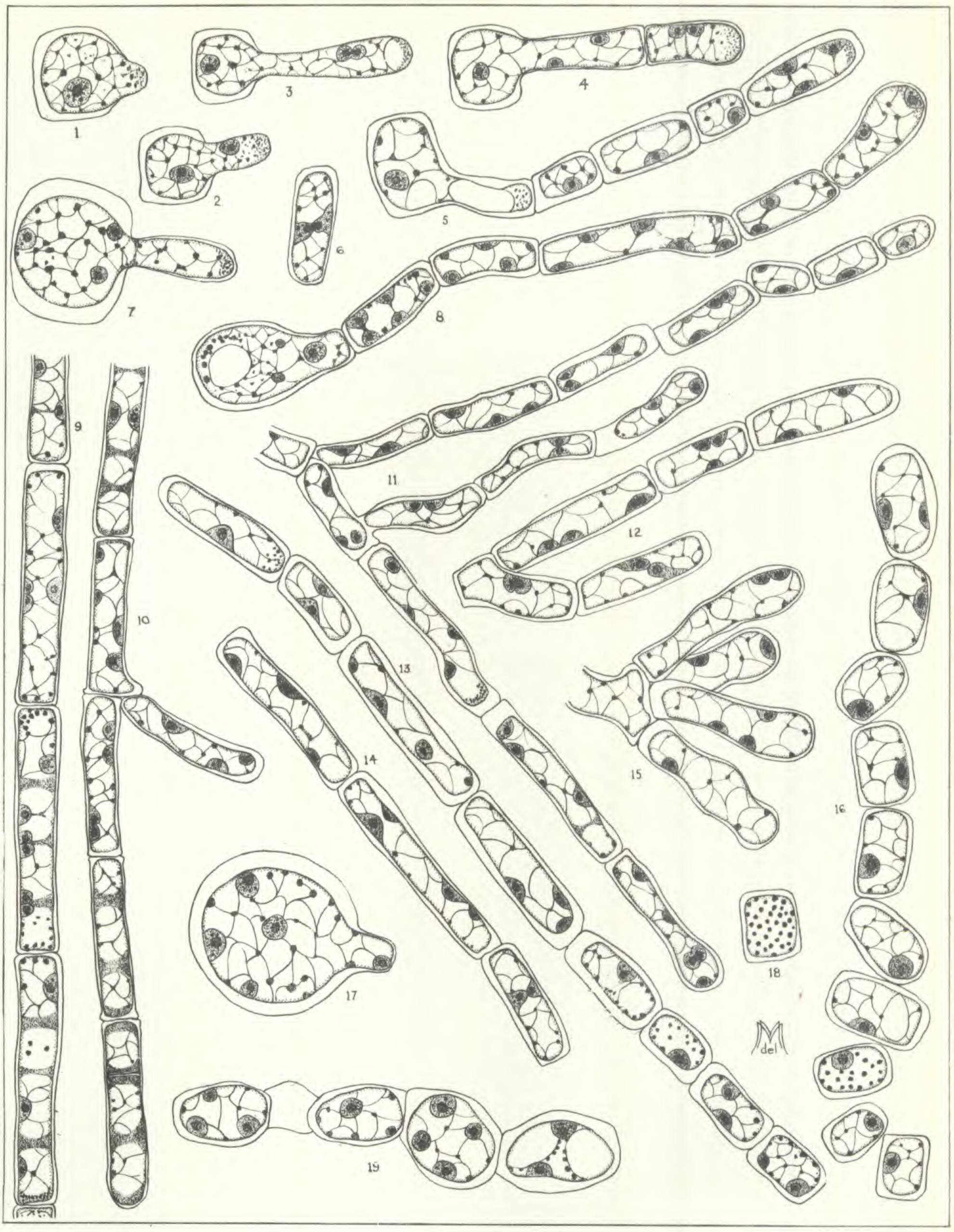
EXPLANATION OF PLATE

PLATE 7

Geotrichum versiforme

All figures drawn as correctly as possible with the aid of a camera lucida. All grown on Sabouraud's glucose agar. Figure 10 drawn at × 1440, all others drawn at × 2300; plate reduced approximately one-half.

Figs. 1–4. Germinating arthrospores.
Fig. 5. Cells formed from a germinating arthrospore.
Fig. 6. Arthrospore showing type of nuclear division.
Fig. 7. Germinating chlamydospore.
Fig. 8. Advanced stage of fig. 5, showing cell formation and multinucleate condition.
Figs. 9–10. Coenocytic cells with the beginning of cross-wall formation.
Figs. 11–12. Branching hyphae showing young coenocytic cells becoming uninucleate arthrospores.
Fig. 13. Filament of cells with arthrospore formation.
Fig. 14. Cells showing nuclear condition and division.
Fig. 15. Branching condition.
Fig. 16. Chain of arthrospores.
Fig. 17. Germinating chlamydospore.
Fig. 18. Arthrospore showing granular appearance of inner wall.
Fig. 19. Chain of cells, probably chlamydospores.



MOORE—GEOTRICHUM VERSIFORME

COCKAYNE, BOSTON

EXPLANATION OF PLATE

PLATE 8

Geotrichum versiforme

All figures drawn as accurately as possible with the aid of a camera lucida. Figures 25–27 drawn at × 1440, all others drawn at × 2300; plate reduced approximately one-half.

Figs. 20–21. Coenocytic cells, probably chlamydospores, on Sabouraud's glucose agar.

Fig. 22. Branching condition of mycelium submerged in Sabouraud's maltose agar.

Fig. 23. Large round chlamydospores on the same medium.

Fig. 24. Mycelium on the same medium.

Figs. 25–27. Whole cells mounted in neutral red, showing volutin content.

Figs. 28–32. Cells mounted in iodine potassium iodide, showing glycogen as the heavily stained material, lipoidal substances as small granular hyaline bodies, and probable chondriosomes as small dark bodies.

Figs. 33–41. Living cells mounted in distilled water to which was added a pinch of benzidine sulphate, showing vacuoles with dancing bodies, probably volutin.

Figs. 42–48. Living cells mounted in 2 per cent osmic acid, showing fat content.

Figs. 49–53. Cells mounted in 5 per cent platinic chloride, showing fat content.